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Hydrolysis of Proteins for Amino Acid Analysis*

Recent collaborative studies on the quantitative determination of amino acids by microbiological assay (1) and also by ion-exchange column chromatography (2) have emphasized anew the usefulness and dependability of both methods when applied to known mixtures of amino acids. A continuing problem in the amino acid analysis of proteins, however, is the preparation of hydrolysates which truly represent the amino acid composition of the unhydrolyzed material, and the problem is even more serious in the analysis of foods and feeds containing relatively little protein. Even a cursory inspection of the literature reveals the multiplicity of conditions that have been used for the preparation of acid hydrolysates of proteins and proteinaceous materials. A useful review of methods has appeared in Block and Weiss' handbook (3) and a shorter summary in the manual of Alexander and Block (4). The great variety of methods proposed for acid hydrolysis is not the result of arbitrary selection of conditions; rather it is a reflection of the effort which has been devoted to the search for an optimal set of conditions which will permit the hydrolysis of all resistant peptide linkages without, at the same time, bringing about extensive destruction of labile amino acids. This may well be impossible for, even under "best" conditions, acid hydrolysis may be expected to result in total disappearance of tryptophan and lesser, variable destruction of cystine, serine, threonine, and other labile amino acids, the extent of which may be more or less important depending on the accuracy required from the analysis.

In the analysis of purified proteins, where highest accuracy is particularly important, it has become common practice in recent years to prepare acid hydrolysates in essentially the same way, that is, in sealed, evacuated tubes, using a large excess of glass-distilled 6*N* HCl and accurately controlled heating at 110° for at least two different periods of time, often 20 and 70 hours. A detailed procedure[1] of this kind, especially designed for chromatographic determination of amino acids by the use of automatic recording equipment, has been described by Moore and Stein (5). It has been proposed that similar conditions be applied to the hydrolysis of foods. Of primary importance in this connection is the use of a large excess of acid, since the amount of humin formed is reduced markedly under dilute conditions (6, 7). A study along these lines was begun by Dustin, *et al.* (8). In this work dilute solutions of synthetic mixtures of 15 amino acids (but not cystine, methionine, and trytophan) were boiled for 22 hours under reflux in 6*N* HCl in both the presence and the absence of carbohydrate. Amino acids were determined by ion-exchange column chromatography. In no instance did the addition of starch or glucose (2 g) to 25–50 mg of amino acids per 200 ml of 6*N* HCl lower the observed recovery of an amino acid by as much as 3%. As the next step in the study it was planned to investigate the recovery of amino acids from proteins of known composition in the absence and presence of carbohydrate during hydrolysis, but this has not been done. Nevertheless, after hydrolysis in the manner just outlined, apparently successful amino acid analyses of such materials as cassava, barley, hay, and linseed oil-cake were made by Moore,

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Bigwood, and their collaborators (9). Because methionine was largely converted to sulfoxide during hydrolysis, final methionine percentages included the amino acid converted to the sulfoxide and determined as a well-separated peak in the chromatograms. Independent analyses were required not only for trytophan but for cystine, which was also destroyed during hydrolysis.

Despite the fact that, at present, no single method of hydrolysis is adequate for complete amino acid analysis of proteins, some useful purpose can be served by standardization of the method of acid hydrolysis. It is planned to investigate the problem in the way suggested by Dustin, *et al.* (8), that is, by studying the effect of added carbohydrate (starch) on the recovery of amino acids from a well-analyzed, purified protein (β-lactoglobulin) when acid hydrolysis is carried out in dilute solutions. Amino acids will be determined by ion-exchange chromatography in an automatic recording analyzer. It is hoped that such hydrolysates will be suitable also for microbiological assay. The determination of tryptophan and cystine, and perhaps methionine, present special problems which will require other studies.

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